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# Biosorption of Copper Zinc and Chromium from Aqueous Solution by Fungal Strains

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## ABSTRACT

Fungi con accumulate heavy metals and radionuclides even from dilute external concentration. Fungal cell wall and their component have a major role in biosorption and also take up suspended metals particles and colloids. The biosorption of Copper, Zinc and Chromium from aqueous solution on to the unmodififide compact biomass of filamentous fungus *Aspergillus niger, Altrnaria solani* and *Rhizopus arrhizus* was studied in the concentration range of 1-20 mM. The effect of temperature, best results were obtained at 25-30°C for fungal strain. *A. niger* was 30°C, the dry weight 172.4 mg while *Altrnaria solani* was 22°C, the dry weight 142 mg. *Rhizopus arrhizus* was 30°C, the dry weight 160 mg after 15 days inoculation. *Aspergillus niger, Altrnaria solani* and *Rhizopus arrhizus* was good found biosorption of Copper, Zinc and Chromium from aqueous solution to be 72 hours at an initial metal concentration. While comparing the percentage uptake, Copper, Zinc and Chromium removal decreased with increasing initial metal concentration. The bioaccumulation of heavy metals by these fungus isolates was observed to be in the order of *Aspergillus niger* > *Rhizopus arrhizus* > *Altrnaria solani.*

**Keywords:** Biosorption, *Aspergillus niger, Altrnaria solani, Rhizopus arrhizus*, Copper, Zinc and Chromium.

## INTRODUCTION

Heavy metals are wide spread Pollutants of great environmental concern as they are non-degradable and thus persistent. It is perceived that there is a permissible

limit of each metal, above which they are toxic (Gupta *et al.,* 2000). Heavy metal releases to the environment are increasing continuously as a result of industrial activities and technological development, posing a significant threat to the environment and public health because of their toxicity, accumulation in the food chain and persistence in nature. It is therefore important to develop new methods for metal removal and recovery from dilute solutions and for the reduction of heavy metal ions to very low concentrations. The use of conventional technologies, such as ion exchange, chemical precipitation, reverse osmosis and evaporative recovery, for this purpose is often inefficient and/or very expensive. Interaction between micro-organism and ions of different metals con be divided in two basic categories; transformation ,that lead to the mobilization of heavy metals ant transformation that immobilize metals such as biosorption or different type of microbial precipitation or binding of metalloids to macromolecules. (Gadd, 2000, Slaninka *et al*., 2006 Simonovicova & Fronkove, 2001), In case of biosorption ion exchange is the most important mechanism that is realized by interaction between ion of metals and active groups on cell wall biopolymer. The biomass represents polyelectrolyte with amino, carboxyl, phosphate, phenol and sulphydriall active groups (Naza *et al.*, 2005).

Fungi con accumulate heavy metals and radionuclides even from dilute external concentration. fungal cell wall and their component have a major role in biosorption and also take up suspended metals particles and colloids (Ahmad *et al.*, 2006), fungi con be grown in substantial amount using unsophisticated fermentation techniques and expensive growth media (Preetha and Virathagiri, 2005). Therefore, bioaccumulation carried out by fungi could serve as economical means of treating metals containing effluent. (Cabuk et *al.*, 2005) the removals of Zn, Cr and Cu from aqueous solution on to the unmodififide compact biomass of filamentous fungus *Aspergillus niger, Altrnaria solani* and *Rhizopus arrhizus*, was studied in the concentration range of 1-20 mM. Non living biomass of *Aspergillus fumigatus* RHO5 and Aspergillus. *flavus* RHO 7 have been show to absorb more then 80% Zn from aqueous solution (Faryal *et al*., 2006). Many fungi and yeast have shown an excellent potential of metal biosorption, particularly the genera *Rhizopus*, *Aspergillus*, *Streptoverticillium* and *Saccharomyces* (Volesky *et al.,* 1981).

The present study was carried out to evaluate potential fungus for use in bioremediation (biosorption) of Copper Zinc and Chromium, a heavy metal present in effluents from various industries. Fungal from waste water and soil contaminated with industrial effluent was first assessed fir endurance of Copper Zinc and Chromium by determination of maximum resistance level of these fungal strains.

## METHODS AND MATERIALS

### Isolation of microorganisms from polluted sites

Water sample were collected in sterilized glass bottles, and analyzed with in 8 hrs. The water samples were enumerated for microorganisms employing a serial dilution technique. Water samples were

serially diluted (10-to 10 000-fold). Aliquots of 100 μl of different dilution were plated on PDA (potato dextrose Agar) plates (three replicates) to ensure the growth of microorganisms present in samples. After at least 3 days of incubation at 25º C, developed colonies were randomly picked and isolated. The results obtained on PDA. Purified isolates were obtained by single spore technique (Dickson, 1933) or hypal tip culture (Brown, 1924) repeatedly colonies in PDA medium and observation under light microscopy.

Pure cultures of isolated micro-organisms were identified using the keys of Ellis *et al.,* (1971); Ellis *et al*, (1976); H.L. Barnett *et al*. (1972) and Joseph. C. Gilman (1998). The cultures were characterized to the genus level on the basis of macroscopic characteristics (colonial morphology, colour and appearance of colony, shape) and microsocopic characteristic (septation of mycelium, shape, diameter and texture of conidia).

**Effect of temperature**

In order to observe the effect of temperature on the biomass of fungal strain, potato dextrose broth (PDB) media was used as the growth media. Each flask was inculcated with one ml of spore suspension ($10^8$ spore/ml) and keeping them at seven different temperatures (10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C) in the incubation for 7 days. After 7 days of growth the biomass of the fungal isolate of fungal strains was harvested on sterile filter paper (Whatman no. 1) and oven dried at 70°C for 72 hours. Mycelia dry biomass of each strain was recorded.

**Screening and selection of heavy metal-resistant microorganisms**

Purified isolates were screened on the basis of their tolerance to Copper, Zinc and Chromium. A disk of mycelium was inoculated aseptically on PDA plates supplemented individually with 1mM of heavy metal. The metals salts used were Potassium dichromate, Zinc sulfate, and Cupric sulfate. The inoculated plates were incubated at 25º C for at least 7 days. The effect of the heavy metal on the growth of the isolates tested was estimated by measuring the radius of the colony extension (mm) against the control (medium without metal) and the determination of the index of tolerance. The index is defined as the ratio of the extension radius of the treated colony to that of the untreated colony. Isolates showing resistance to Copper, Zinc and Chromium were selected for the following experiments.

**Determination of minimum inhibitory concentration (MICs)**

The resistance of the selected isolates to Copper, Zinc and Chromium was determined by the dilution method. Metal ions were added separately to PDB medium at concentration of 1 to 20 mM. The flasks were inoculated with 8 mm agar plugs from young fungal colonies, pre-grown on PDA. Three replicates of each concentration and controls without metal were used. The inoculated flasks were incubated at 25ºC for at least 30 days. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of metal that inhibit visible growth of the isolates.

## Analytical methods

The samples (5 mL) were drawn and digested for metal analysis,along with biomass which was filtered, dried at 55°C and weighed, at the same time dailyFrom the initiation to the termination of the study, as described earlier (Faryal *et al.*, .,2006). Residual lead concentrations left in these samples were measured by using airacetyleneflame of Solar Unicam atomic absorption spectrophotometer at 283.3 nm.determination limit for Cr was 0.05 mg/L, sensitivity 0.05 mg/L and optimum concentration range 1-20 mg/L (Clesceri *et al.*, ., 1989). All biosorption experiments were carried out in duplicate and values used in calculation were arithmetic averages of experimental data. The amount of biosorbed per gram of dried biomass was calculated using the following formula:

$$Q = (C_i - C_e) \times V/m$$

Where

Q = metal ions (mg) biosorbed per gram of biomass,

Ci = initial metal ion concentration (mg/L),

Ce = final concentration of metal ion (mg/L),

m = dry weight of biomass in reaction mixture (g)

V = volume (L) of the reaction mixture (Çabuk *et al.*, 2005; Khani *et al.*, 2006).

## Statistical analysis

Data are represented as Mean ± Standard Error of Mean. Data were subjected to statistical analysis through Student's 't'-test, as described by Steel & Torrie (1960).

## RESULT AND DISCUSSION

Fungi isolated from contaminated sites were identified as *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata*, *Alternaria solani*. *Rhizopus arrhizus*, *Rhizopus* sp., *Penicillium* sp. and *Fusarium* sp**.** While comparing the effect of temperature, best results were obtained at 25-30°C for fungal strain. *A. niger* was 30°C, the dry weight 172.4 mg while *Altrnaria solani* was 22°C, the dry weight 142 mg. *Rhizopus arrhizus* was 30°C, the dry weight 160 mg after 15 days inoculation.

In this paper was determined the ability of fungal *Aspergillus niger*, *Altrnaria solani* and *Rhizopus arrhizus* to biosorb (immobilize) oxyanions of from aqueous solutions. The concentration of Cu, Zn and Cr in the water sample was found to be above the permissible limits of 0.05, 5 and 0.01 ppm, Copper is a co-factor in numerous enzymatic processes and represents the third most abundant transition metal found in living organisms (Brandolini *et al.*, 2002 ). The all the three fungi tested were able to grow at a copper concentration of 7.5 to 10 mM or higher *Aspergillus niger* was more tolerant for Cu. The MICs for Cu was in the range of 20 to 25 mM in *Aspergillus niger* , 10 to 15 mM in *Alternaria alternata* and 7.5 to 10 mM in *Rhizopus arrhizus*. Michael S. Price (2000) reported the *Aspergillus niger* was able to grow on plates amended with copper at a level five times that inhibitory to the growth of *Saccharomyces cerevisiae* and *Aspergillus niger is* capable of removing 91% of the copper from treated swine effluent. Copper resistance in *Aspergillus niger* is due to an active process involving copper metallothionein synthesis (Kermasha *et al.*, 1993).

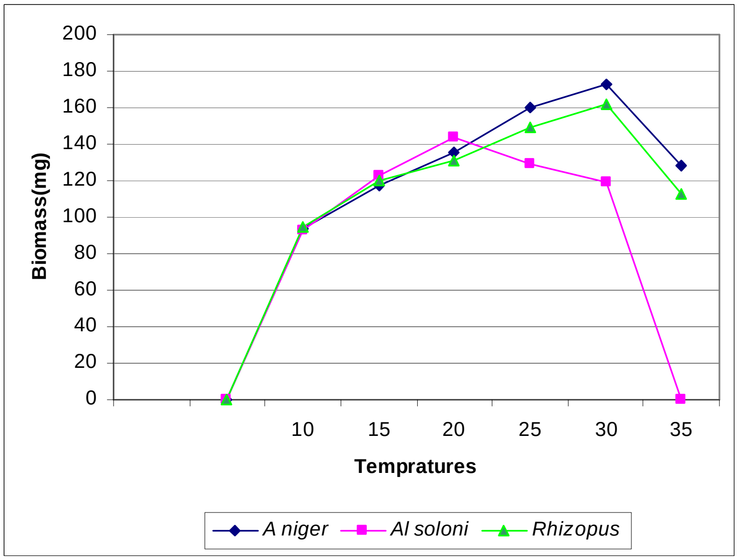


**Figure 1: Growth of fungal strain as Aspergillus *niger, Alternaria solani* and *Rhizopus arrhizus*, in different temperatures.**

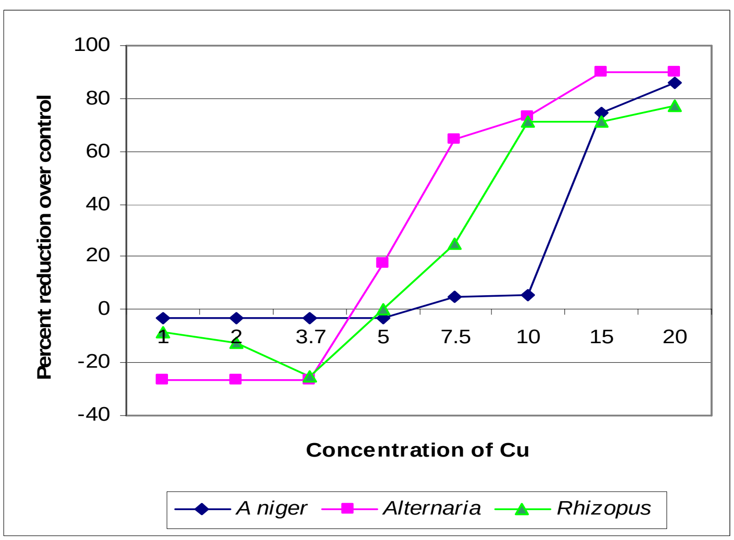


**Figure 2: Showing Comparative analysis of Cu tolerance by *Aspergillus niger Alternaria alternata* and *Rhizopus arrhizus*.**

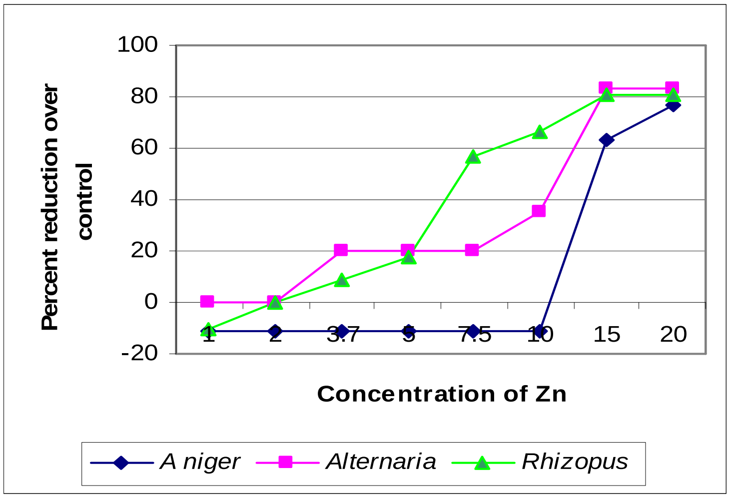


**Figure 3: Showing Comparative analysis of Zn tolerance by *Aspergillus niger*, *Alternaria alternata* and *Rhizopus arrhizus*.**

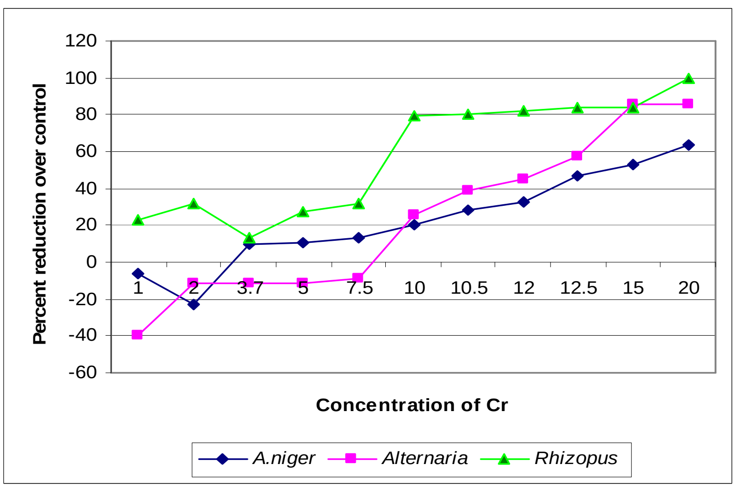


**Figure 4: Showing Comparative analysis of Cr tolerance by *Aspergillus niger*, *Alternaria alternata* and *Rhizpous arrhizus*.**

Zinc is essential for all organisms, although at high concentration it can be toxic (Balsalobre *et al.*, 2003). The Zinc MICs were in the range 15 to 20 mM in *Aspergillus niger* 15 to 20 mM in *Alternaria alternata*, 7.5 to 15 mM in *Rhizopus arrhizus* respectively.The fungal colour and morphology were both affected by high Zn concentrarion. de Rome and Gadd (1987) reported *Rhizopus* arrhizus was able to remove approximately 130 moles zinc per gram fungal dry weight. Luef *et al.*, (1991)

found *Aspergillus niger* was to be superior to *Penicillium chrysogenum* and *Claviceps paspali* in its ability to remove zinc form solution. Levinskaite (2001) demonstrated that the growth rate of *Penicillium atratmentosum* 25SL decreased slowly as the Zn ions concentration increased up to 40 mM. Michael S. Price (2000) reported the *Aspergillus niger* was able to grow on plates amended with copper at a level five times that inhibitory to the growth of *Saccharomyces cerevisiae* and *Aspergillus niger* is capable of removing 70 % of the zinc from treated swine effluent. Similar results were reported by Vadkertiova and Slavikova (2006), who found that *Pichia anomala*, *Candida krusei* and *Cryptococcus laurentii* tolerated high concentrations of zinc (up to 20 mM).

Hexavalent chromium is the toxic form of chromium released during industrial processes such as leather tanning and pigment manufacture (Srivastava and Thakur, 2006). The most sensitive isolate belonged to the genus *Rhizopus* with MICs of 7.5 to 10 mM. *Alternaria alternata* and *Aspergillus niger* isolates were also tolerant to chromium up to 10 to 15 mM in *Alternaria alternata* and *Aspergillus niger* was most tolerant for chromium above MICs 20 to 25 mM. . Our results were comparable with those reported by Badar *et al*. (2000), Verma *et al.,* (2001), Bai and Abraham (2003), Malik (2004), Zouboulis *et al.,* (2004).

Metals such as copper and zinc are essential to biological actions, however, all metals, whether essential and inessential will tend to show toxicity at certain levels. Their toxicity may be presented differently, depending on the isolate and its site of isolation. However, although some authors found that microorganisms isolated from contaminated sites were more tolerant than those from natural environments (Massaccesi *et al.,* 2002; Malik, 2004). In this way isolates originating from highly metal contaminated sites (Mohan Meakins) have shown a comparatives metal resistance to those isolated from uncontaminated sites. Thus, the presence of metal may have acted as a selective pressure for metal resistant fungi. Our findings indicate that fungal populations isolated from heavy metal contaminated sites have the ability to resist higher concentration of metals. *Aspergillus niger* was the most resistant to all the metals tested, which may make them promising candidate for further investigations regarding their ability to remove metals form contaminated environments.

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